Collective oscillations in microtubule growth

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Large groups of microtubules are observed to undergo coherent oscillations in length. This process is suspected to be important to the mechanism of cell division. We propose a model, related to the bounded-unbounded transition of microtubules proposed by M. Dogterom and S. Leibler [Phys. Rev. Lett. **70**, 1347 (1993)], which is in good agreement with experiments. [S1063-651X(96)04306-1]

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Collective oscillations in out-of-equilibrium systems are fascinating problems, and were subject to intense studies in the past decade. These oscillations have been observed in many biological systems, which are known to be far from equilibrium. An important example is the growth of groups of microtubules, which show oscillations during *in vitro* experiments. These oscillations are suspected to play a role during the cell cycle [2].

Microtubules (MTs) are long, rigid polymers made of $\alpha-\beta$ tubulin dimers, and form a great part of the cytoskeleton of all eukaryotic cells. They are essential for transport phenomena in the cell, where they are used as rails by molecular motors like dynein. They also play a crucial role during cell division, when the microtubule network is dismantled and assembled again in a short lapse of time. This last behavior is believed to be related to the so-called *dynamic instability*, first observed by Mitchison and Kirshner [3], and was intensively studied during the last decade (for a review, see [4]). The term dynamic instability means that under certain circumstances, MTs switch randomly between a growing (+) and a shrinking (−) state. In the (+) state, MTs adsorb free GTP-tubulin from solution and increase in length. Later on, this GTP-tubulin is hydrolyzed and transformed into GDP-tubulin. (GTP stands for guanosine triphosphate and GDP for guanosine diphosphate.) When a MT switches to the (−) state, it loses its GDP-tubulin and decreases in length. The exact nature of the transition is not known, but it has been proposed that there is a stabilizing cap of GTP-tubulin on the ends of (+)-MTs. When this cap is lost by fluctuations, MTs switch to the (−) state and shrink until the capture of a new stabilizing cap. The existence of this cap is not yet proved. The dynamic instability has been observed both *in vitro* and *in vivo* [5–7].

During a typical *in vitro* experiment, tubulin and GTP are added in a buffer at 4 °C. When the temperature is changed to 37 °C, MTs nucleate and begin to grow. The mean length of MTs (or the total density of polymer), grows in a monotonic fashion before reaching a plateau. However, when the initial concentration of tubulin is high enough and when there exists some mechanism to regenerate GTP-tubulin (which can be achieved by an excess of free GTP or by addition of some enzymes), the polymer density oscillates with time (see Fig. 1), and the amplitude of oscillation can become very large [8–11]. This means that individual MTs synchronize themselves and switch between (+) and (−) states in a coherent way. This behavior is quite similar to the well known Belousov-Zhabotinsky oscillations. These oscillations can provide a biological clock at the molecular scale for the cell (for a review, see [2]). Many numerical simulations were performed by different authors [9,12,13] to explain these oscillations, but the results are, until now, unsatisfactory. The most complete work to our knowledge is the one performed by Marx and Mandelkow [13], where they conclude that the basic ingredients of dynamical instability are not by themselves sufficient to explain smooth, large scale oscillations observed in experiments.

We show in this paper that the dynamical instability alone is sufficient to explain oscillations: recently, Dogterom and Leibler [1] proposed a simple statistical model for MT dynamics, and they pointed out a bounded growth to unbounded growth transition in MTs (see below), observed by several authors [14,15]. We will show that this model, modified to take into account the GTP-tubulin consumption can give rise to large sustained oscillations, as observed in experiments.

Let us present the model. Following [1], we denote

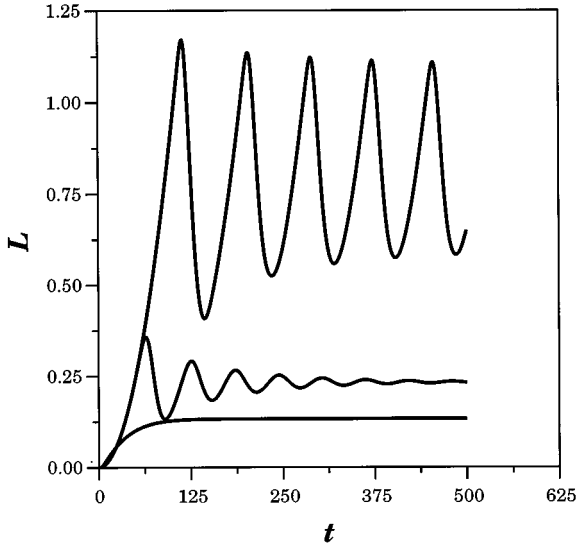

FIG. 1. A typical numerical solution of evolution equations. $v_+=0.1$, $v_-=0.4$, $\nu=0.01$, $f_{-+}=0.01$, $f=0.1$, $c_T^*=100.9$, $\alpha=0.05$, $\beta=3$, $\gamma=83.3$. Lengths are measured in μm and time in seconds. Concentration are in μM. Order of magnitude of f_i, v_i obtained from Ref. [14]. Bottom curve: $c_0=95$; middle curve: $c_0=110$; top curve: $c_0=150$.

$p_{\pm}(z,t)$ the probability density for finding, at time t, a MT in the $\pm$ state with a length between z and $z+dz$. We can write the detailed balance equations

$$\partial_t p_+ = -f_{+-}p_+ + f_{-+}p_- - v_+\partial_z p_+, \tag{1}$$

$$\partial_t p_- = f_{+-}p_+ - f_{-+}p_- + v_-\partial_z p_-, \tag{2}$$

where f_{+-} is the catastrophe [switch from $(+)$ to $(-)$] frequency, f_{-+} the rescue [switch from $(-)$ to $(+)$] one, v_+ the MT speed in growing and v_- in shrinking state.

These equations have to be supplemented by boundary conditions. Two cases can be considered: (i) Nucleation on stable centrosomes; (ii) spontaneous nucleations. In case (i), the probability of empty centrosomes is denoted by $s(t)$ and the rate of nucleation by ν. Then one has [1]

$$\partial_t s = -v_+ p_+(0,t) + v_- p_-(0,t), \tag{3}$$

$$\nu s = v_+ p_+(0,t). \tag{4}$$

In case (ii), given spontaneous nucleation at rate $\overline{\nu}$ one simply has

$$\overline{\nu} = v_+ p_+(0,t). \tag{5}$$

This can be formally considered as the limit of centrosome nucleation when $s\to\infty$, $\nu\to 0$ and $\nu s = \overline{\nu}$ (note that in this case, $p_{\pm}$ has to be considered as the concentrations of MTs with their length between z and $z+dz$ and s refers to the concentration of empty sites).

The above equations have a steady state solution $p_{\pm} = A_{\pm}\exp(-z/\ell)$, where

$$\ell = \frac{v_+ v_-}{v_- f_{+-} - v_+ f_{-+}}, \tag{6}$$

$$A_+ = \frac{1}{\ell(1 + v_+/v_-) + v_+/\nu}, \tag{7}$$

$$A_- = A_+ v_+/v_-. \tag{8}$$

[In the case of spontaneous nucleation, Eq. (7) has to be changed to $A_+ = \overline{\nu}/v_+$.] As pointed out by Dogterom and Leibler, this solution does not exist when $v_+ f_{-+} > v_- f_{+-}$. In this latter case, MTs grow in an unbounded regime: Their average length increases linearly in time and their length distribution becomes a moving Gaussian in the long time limit.

A remark should be made at this point. As demonstrated by many experiments, all stochastic parameters (f_i, v_i, ν) depend on the GTP-tubulin (GTP-TU) concentration c_T. So, a variation in c_T can provoke a transition between a bounded and an unbounded growth regime. The key point of our oscillation model is here: We suppose a dependence of stochastic parameters on c_T such that there exists a critical concentration of GTP-TU, c_T^*, separating an unbounded growth $(c_T > c_T^*)$ and a bounded growth $(c_T < c_T^*)$ regime. If at the initial time, $c_T(t=0) < c_T^*$, MTs grow in a monotonic fashion to reach a plateau, which corresponds to the steady state solution (see below). If, however, $c_T(t=0) > c_T^*$, the MTs grow rapidly in an unbounded regime. As they increase their length, GTP-TU is consumed, c_T becomes smaller than c_T^* and MTs mean length decreases to reach the length ℓ of the steady state solution. After a certain time, free GTP in the solution regenerates GTP-TU from GDP-TU produced by shrinking MTs, c_T then becomes greater than c_T^* and the growth resumes. This cycle can be repeated many times. The transition between the bounded and the unbounded regime gives the system extreme sensitivity to small changes in parameter values, and it can be sufficient to induce sustained oscillations.

Let us complete dynamical Eqs. (1)–(4) to take into account (i) the consumption of GTP-TU by growing MTs; (ii) the production of GDP-TU by shrinking MTs; (iii) the regeneration of GTP-TU from GDP-TU by the action of free GTP in the solution (GTP + GDP-TU → GDP + GTP-TU). As the adsorption of GTP-TU by $(+)$ MTs and the release of GDP-TU by $(-)$ MTs take place only at the ends of MTs, they are respectively proportional to the total number of growing and shrinking MTs. Then, kinetic equations for the concentrations c_T and c_D of GTP-TU and GDP-TU simply read

$$\partial_t c_T = -\gamma v_+ \int_0^\infty p_+(z,t)\,dz + \alpha c_D, \tag{9}$$

$$\partial_t c_D = \gamma v_- \int_0^\infty p_-(z,t)\,dz - \alpha c_D, \tag{10}$$

where $\gamma = c_{mt}/a$ in the case of nucleation on centrosomes (with c_{mt} the centrosome concentration) or $1/a$ in the case of spontaneous nucleation; a is the length of MTs units ($\approx$6 Å); α is a phenomenological parameter corresponding to the regeneration rate of GTP-TU from GDP-TU (either due to the presence of an excess of free GTP or to enzymatic process) which will be assumed to be time independent. In the case of regeneration from free GTP, α actually depends on the concentration of free GTP (c_{free}), but when c_{free} largely exceeds the tubulin concentration (as in most experiments), α can be assumed to be approximately constant over the interesting time scale. In the above expressions, we have neglected spatial dependence of the concentrations c_T and c_D, assuming a fast diffusion of species. We will discuss this point further. As the total number of tubulin dimers in the solution is conserved during the growth and is equal to the initial number of tubulin c_0 put in the solution, we also have a conservation equation

$$c_T + c_D + \gamma L = c_0, \tag{11}$$

where $L = \int_0^\infty z[p_+(z,t) + p_-(z,t)]\,dz$ is the mean length of MTs.

Taking into account the dependence of dynamical parameters on c_T, we search again for a steady state solution of Eqs. (1)–(3), (9)–(11). The expressions given by Eqs. (6)–(8) remain valid. Using these solutions in Eqs. (9)–(11), we obtain a self-consistent solution for c_T

$$c_T - c_0 = -\gamma\left[\frac{v_+}{\alpha}\ell A_+ + \ell^2(A_+ + A_-)\right]. \tag{12}$$

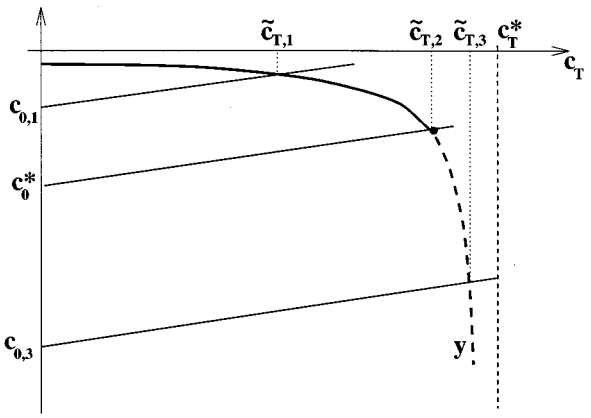

FIG. 2. The graphical solution of the equation $c_T - c_0 = y(c_T)$, for various values of c_0. $y(c_T)$ is the right hand side of Eq. (12). The dashed part of y corresponds to unstable solutions.

Note that when $c_T \to c_T^*$, $\ell^2(A_+ + A_-) \to \infty$, so that Eq. (12) always has a solution. The graphical solution of this equation is shown in Fig. 2.

The time-dependent Eqs. (1)–(3), (9)–(11) cannot be solved analytically, and we have to use numerical methods. Figure 1 shows a typical numerical solution of our model, for increasing values of the control parameter c_0 (the initial tubulin concentration). At $t=0$, all MTs have zero length and $c_T = c_0$. For simplicity, we assume that only one of the dynamical parameters, say f_{+-}, is c_T dependent. This choice was motivated by the work of Walker *et al.* [16] who report a strong dependence of f_{+-} on tubulin concentration. The explicit form of f_{+-} we take reads

$$f_{+-}(c_T) = f\{1 - \tanh[(c_T - c_1)/\beta]\}. \tag{13}$$

This particular choice is rather arbitrary, but it is not essential: the only fundamental ingredient is the critical dependence of ℓ on c_T near c_T^*.

As shown in Fig. 1, for weak values of c_0, ($c_0 < c_T^*$), the growth is monotonic, and reaches a plateau after some time. For $c_0 > c_T^*$, damped oscillations appear. The values of final plateau correspond to the solutions of Eq. (12). Finally, when c_0 becomes greater than a critical value c_0^*, stable oscillations are observed. There is a clear transition between a steady state and an oscillatory one, which corresponds to a Hopf bifurcation. To study this transition, we have performed a marginal stability analysis. Denoting $\tilde{c}_T$ the solution of Eq. (12), we have computed the temporal behavior of a small perturbation $c_T = \tilde{c}_T + \epsilon c \exp(\Omega t)$. If the amplification rate $\mathrm{Re}(\Omega)$ is positive, the steady state solution is unstable. For $\nu \gg f_{-+}, f_{+-}$, the complex frequency Ω was found to be a solution of [17]:

$$\frac{\Omega(\Omega+\alpha)}{v_+\Omega/(v_+ + v_-) + \alpha} = \frac{f'_{+-}\gamma v_-}{\Omega + f_{+-} + f_{-+} + (v_- - v_+)/\ell} \times \left(1 - \frac{1}{\ell q}\right), \tag{14}$$

where $f'_{+-} = \partial f_{+-}/\partial c_T|_{c_T = \tilde{c}_T}$ and q (which is an implicit function of Ω) is the $\mathrm{Re}(q) > 0$ root of the dispersion relation corresponding to the free evolution of MTs [Eqs. (1), (2)]:

$$-v_+ v_- q^2 + [\Omega(v_- - v_+) + f_{+-}v_- - f_{-+}v_+]q + \Omega^2 + \Omega(f_{+-} + f_{-+}) = 0. \tag{15}$$

By numerically solving Eq. (14), one can easily check that there exists a critical value c_0^*, equal to that found by numerical resolution of the whole evolution equations. $\mathrm{Re}(\Omega)$ changes its sign and becomes positive when $c_0 > c_0^*$. The oscillation frequency near the transition is given by $\mathrm{Im}(\Omega)$. (A similar result is also obtained in the case of spontaneous nucleation [17].)

Let us now compare some predictions of the present model to experimental results. In a typical experiment, $L(t)$ is studied for various initial tubulin concentrations, c_0. Our model is able to reproduce quite well the experimental results using realistic values of the parameters. In particular, the three different regimes (monotonic, damped, and sustained oscillations) appear quite naturally (see Fig. 1). It is interesting to note that the range of initial concentration c_0 for which oscillations are observed lies in the range of unbounded growth according to [15].

Carlier *et al.* [9] have also studied oscillations for various values of free GTP concentration c_{free}, fixing c_0 to a high value. For weak value of c_{free}, $L(t)$ shows a maximum, and then comes back to a small value. As c_{free} increases, oscillations appear. On the other hand, results published by Wade *et al.* [18] showed a monotonic increase of MTs mean length in the presence of large amount of free GTP in addition to an enzymatic regenerating of GTP-TU. In our model, c_{free} does not appear explicitly, but as a matter of fact, it controls the restitution rate of GTP-TU from GDP-TU, α (α increases with c_{free}). Suppose $c_0 \gg c_T^*$, and α is weak. At time $t=0$, MTs are in the unbounded regime and their lengths begin to increase rapidly. During this growth, they consume GTP-TU and after a time t_1, c_T becomes smaller than c_T^*. The regime switches to the bounded one, and the mean length of MTs decreases to reach the equilibrium value. As α is weak, there is a little GDP-TU to GTP-TU transformation, not sufficient to switch back to the unbounded growth: the mean length reaches a plateau and remains constant. For larger value of α, c_T can reach again c_T^* after the first maximum occurs and oscillations reappear. On the other hand, for very large values of α (much larger than other characteristic frequencies of the model), the oscillating regime disappears again. The regeneration of GTP-TU is so fast in this latter case, that it prevents a great decrease of the MTs mean length. Numerical solutions of the model for increasing values of α are shown in Fig. 3. The appearance and disappearance of the oscillatory instability can also be obtained through the linear stability analysis [Eq. (14)]. These results are in good agreement with the above mentioned experiments and unify the apparently contradictory results published by different authors.

Note that our model can clearly distinguish the role played by the parameters c_0 and α, in particular, that the oscillating regime occurs only for a limited range of α. A

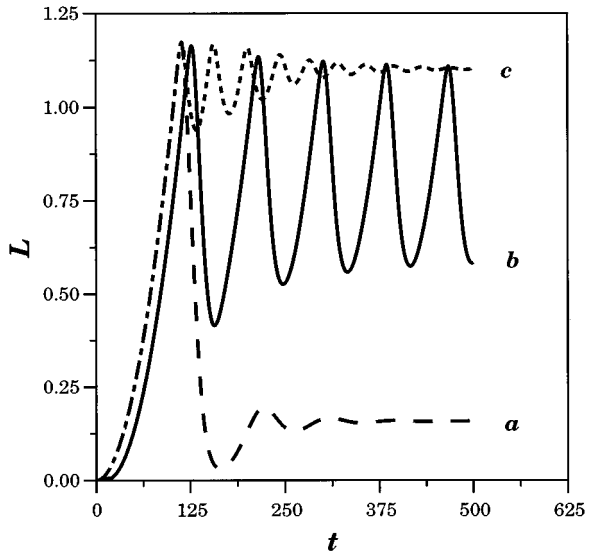

FIG. 3. Numerical resolution of evolution equations for various values of α. Parameters are those of the top curve of Fig. 1, except for α. a: $\alpha=0.005$; b: $\alpha=0.05$; c: $\alpha=0.5$.

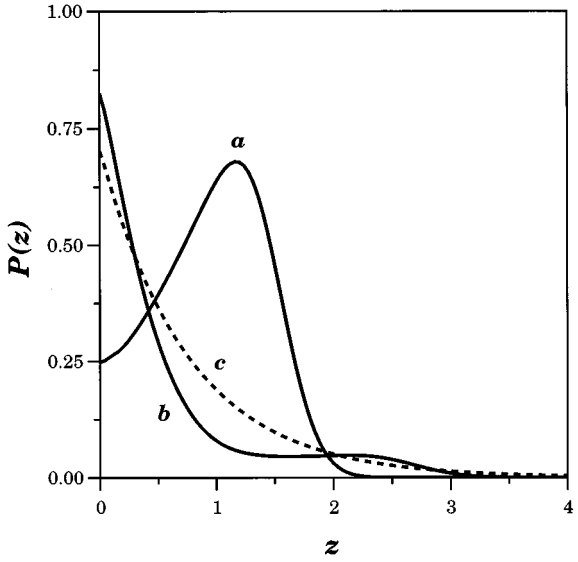

FIG. 5. Population distribution at different times during oscillatory growth. Parameters are those of the middle curve of Fig. 1. (a): $t=66.7s$ (first maximum); (b): $t=91.4s$ (first minimum); (c): $t=500s$ (final state).

schematic diagram which summarizes the various regime of growth as a function of c_0 and α is shown in Fig. 4.

The numerical resolution of evolution equations also allows us to study the time-dependent length distribution of MTs When oscillations appear, the length distribution of MTs $P(z)=p_+(z)+p_-(z)$ is an asymmetric Gaussian at times corresponding to maxima of $L(t)$, which is the signature of an unbounded growth. At times for which $L(t)$ is minimum, a great proportion of MTs are in the $(-)$ state and $P(z)$ has a more complicated shape, intermediate between an exponential and a Gaussian. Finally, when oscillations are damped, $P(z)$ displays the exponential behavior corresponding to the steady state solution. Figure 5 shows these distributions, for the growth corresponding to the middle curve of Fig. 1. This captures the qualitative behavior observed by [9].

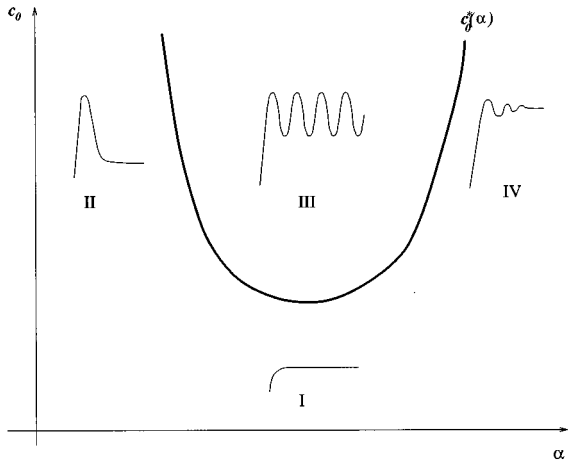

FIG. 4. Schematic diagram of different types of growth as a function of the initial concentration of tubulin c_0 and the restitution rate of GTP-TU α. (I): monotonic growth; (II): damped oscillations and stabilization at a low polymer density; (III): sustained oscillation; (IV): damped oscillations and stabilization at high polymer density. The solid line corresponds to a Hopf bifurcation.

Before concluding, let us discuss one approximation of our model. Here, the nucleation rate of MTs has been considered as constant. As shown by several authors (see [19] and references therein), in the spontaneous nucleation case, $\overline{\nu}$ is srongly c_T dependent. This dependence will change the amplitude and increase the period of oscillations. However, we have checked numerically that the behavior of our model does not change notably when an explicit dependence of $\overline{\nu}$ on c_T is taken into account and this factor alone cannot explain sustained oscillations [17].

In conclusion, we have presented a minimal model of the MTs to explain collective oscillations during *in vitro* growth. This model does not take into account many features and details. For example, when MTs shrink, they release oligomers of GDP-TU which are broken later to free GDP-TU. The chemical equation we used to modelize GDP-TU to GTP-TU transformation is thus a very simplified one, but it can be modified to take into account a more complete description of MTs growth. But this will hide the key point of our model: the MTs synchronization mechanism is controlled by the bounded to unbounded transition.

Previous work [13] concluded that to explain oscillations, one has to add extra parameters to the ingredients of dynamic instability, or to take into account a memory effect (long cap model). We showed here that a simple statistical model such as that proposed by Dogterom and Leibler [1] is sufficient to account for oscillations and there is no need to add any extra parameter. The bounded–to–unbounded transition provides the extreme sensitivity of MTs to small GTP-TU concentration variation. One can note that the synchronization of MTs oscillations in the whole space is a consequence of the assumed homogeneity of the tubulin concentration. Actually, the diffusion lengths are finite. This may induce dephasing of oscillations in different regions of space

and may lead to pattern formation, as in the classical Belousov-Zhabotinsky scenario. Moving and stationary patterns have actually been observed during MT growth [20,21]. It would be interesting to extend the present model to take into account the spatial diffusion of species in order to explain spatial inhomogeneities and morphological bifurcations.

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